APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Sept. 2003, p. 5269–5274 Vol. 69, No. 9
0099-2240/03/$08.00+0 DOI: 10.1128/AEM.69.9.5269–5274.2003
Copyright © 2003, American Society for Microbiology. All Rights Reserved.

Diversity of *Bacillus thuringiensis* Strains from Latin America with Insecticidal Activity against Different Mosquito Species

Jorge E. Ibarra,[1] M. Cristina del Rincón,[1] Sergio Ordúz,[2] David Noriega,[2] Graciela Benintende,[3] Rose Monnerat,[4] Leda Regis,[5] Cláudia M. F. de Oliveira,[5] Humberto Lanz,[6] Mario H. Rodriguez,[6] Jorge Sánchez,[7] Guadalupe Peña,[7] and Alejandra Bravo[7]*

CINVESTAV IPN, Irapuato 36500, Guanajuato,[1] and Instituto Nacional de Salud Publica, Centro de Investigación sobre Enfermedades Infecciosas, Cuernavaca 62508,[6] and Instituto de Biotecnología UNAM, Cuernavaca 62250,[7] Morelos, México; Corporación para Investigaciones Biologicas, 7378 Medellín, Colombia[2]; Instituto de Microbiología y Zoología Agrícola-INTA, Buenos Aires, Argentina[3]; and EMBRAPA-Recursos Genéticos e Biotecnologia, Brasília/DF,[4] and Fundação Oswaldo Cruz FIOCRUZ, Recife PE,[5] Brazil

Received 16 April 2003/Accepted 1 July 2003

The characterization of selected *Bacillus thuringiensis* strains isolated from different Latin America countries is presented. Characterization was based on their insecticidal activity against *Aedes aegypti*, *Culex quinquefasciatus*, and *Anopheles albimanus* larvae, scanning electron microscopy, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and plasmid profiles as well as PCR analysis using novel general and specific primers for *cry* and *cyt* genes encoding proteins active against mosquitoes (*cyt1*, *cyt2*, *cry2*, *cry4A*, *cry4B*, *cry10*, *cry11*, *cry17*, *cry19*, *cry24*, *cry25*, *cry27*, *cry29*, *cry30*, *cry32*, *cry39*, and *cry40*). Strains LBIT315, LBIT348, and IB604 showed threefold higher mosquitocidal activity against *A. aegypti* and *C. quinquefasciatus* larvae than *B. thuringiensis* subsp. *israelensis* and displayed high similarities with the *B. thuringiensis* subsp. *israelensis* used in this study with regard to protein and plasmid profiles and the presence of *cry* genes. Strain 147-8906 has activity against *A. aegypti* similar to that of *B. thuringiensis* subsp. *israelensis* but has different protein and plasmid profiles. This strain, harboring *cry11*, *cry30*, *cyt1*, and *cyt2* genes, could be relevant for future resistance management interventions. Finally, the PCR screening strategy presented here led us to identify a putative novel *cry11B* gene.

Vector-borne diseases are major public health problems, and their prevalence has dramatically increased worldwide (1, 12). Dengue and malaria are transmitted to humans through *Aedes* spp. and *Anopheles* spp. mosquitoes, respectively. Bancroftian filariasis and the West Nile virus are transmitted by *Culex quinquefasciatus*. At present, the best control methods for these diseases are based on vector control (1) that is mainly accomplished by using synthetic insecticides. Additionally, mosquitoes have developed resistance to the major chemical insecticide groups. For instance, global DDT spraying to control mosquito populations succeeded for only 8 years, as mosquito resistance appeared (1).

The use of entomopathogenic bacteria *Bacillus thuringiensis* and *Bacillus sphaericus* as biolarvicides is a viable alternative for insect control (12). *B. thuringiensis* produces proteinaceous inclusions during sporulation that are toxic towards insect larvae upon ingestion (15). The parasporal body of *B. thuringiensis* consists of one or more insecticidal δ-endotoxins (named Cry and Cyt) (http://www.biols.susx.ac.uk/Home/Neil_Crickmore/Bt/index.html). These toxins are highly specific, are harmless to humans, vertebrates, and plants, and are completely biodegradable, so no residual toxic products accumulate in the environment (15).

The number of known *B. thuringiensis* strains active on diptera is growing (6, 19). A feature of all mosquitocidal strains is the presence of Cyt toxin, which is not very toxic by itself. The mosquitocidal activity of a *B. thuringiensis* strain is not only the additive effect of each toxin but a complex synergistic interaction among them. *B. thuringiensis* subsp. *israelensis* produces four Cry toxins (Cry4Aa, Cry4Ba, Cry10Aa, and Cry11Aa) and two Cyt toxins (Cyt1Aa and Cyt2Ba) (6), and synergism between these toxins has been reported (4, 18). Interestingly, the presence of the Cyt toxin delays or prevents the development of resistance to Cry toxins (17). *B. thuringiensis* subsp. *israelensis* has been used in the field for nearly 20 years with no development of insect resistance (7, 11).

The search for native strains with activity against dipteran species could have an impact on the control of mosquitoes worldwide. Here, we characterized selected strains from Latin American *B. thuringiensis* collections. The characterization included the identification of mosquitocidal *cry* and *cyt* genes by multiplex PCR analysis with novel general and specific primers. *B. thuringiensis* strains containing some of the previously described *cry* genes as well as potentially novel Cry proteins were identified. Also, *B. thuringiensis* strain 147-8906 showed toxic activity against *A. aegypti* similar to that of *B. thuringiensis* subsp. *israelensis* but showed different gene content. This strain has potential interest for insect resistance management.

* Corresponding author. Mailing address: Instituto de Biotecnología, Universidad Nacional Autonoma de Mexico, Apartado Postal 510-3, Cuernavaca 62250, Morelos, México. Phone: (52-73) 291635. Fax: (52-73) 172388. E-mail: bravo@ibt.unam.mx.

TABLE 1. Characteristics of *B. thuringiensis* strain collections used in this study

Country	No. of *B. thuringiensis* strains	*cry* genes recognized (% of strains if less than 100%)	Toxicity assay results (no. of strains toxic to the indicated genus)	Other assays performed
Brazil	1,378	*cry1* (~90%), *cry2*, *cry3*, *cry4*, *cry8*, *cry10*, *cry11*	229 vs. *Diptera*, 412 vs. *Lepidoptera*, 34 vs. *Coleoptera*	SDS-PAGE
Mexico	683	*cry1*	400 vs. *Diptera*, 425 vs. *Lepidoptera*, 420 vs. *Coleoptera*	SDS-PAGE, plasmid patterns, Western and Southern blot, serotyping, beta-exotoxin
Mexico (reference 3)	690	*cry1* (~49%), *cry3*, *cry7*, *cry8*, *cry9*, *cry11*, *cyt*	100 vs. *Diptera*, 320 vs. *Lepidoptera*, 410 vs. *Coleoptera*	SDS-PAGE, enzyme-linked immunosorbent assay
Colombia	3,780	*cry1* (55%), *cry3*, *cry4*, *cry11*	1,552 vs. *Diptera*, 919 vs. *Lepidoptera*, 1,424 vs. *Coleoptera*	SDS-PAGE

MATERIALS AND METHODS

Bacterial strains. *B. thuringiensis* subsp. *israelensis* HD567 and *B. thuringiensis* subsp. *kurstaki* HD1 were from *Bacillus* Genetic Stock Center (Columbus, Ohio), and *B. thuringiensis* subsp. *jegathesan* was kindly supplied by S. S. Gill (University of California—Riverside, Riverside, Calif.). Other *B. thuringiensis* strains were isolated (16) from soil samples from Mexico, Brazil, and Colombia (Table 1). *B. thuringiensis* strains were grown in M-1 medium (13) at 30°C with agitation at 200 rpm until sporulation was complete. Lyophilized spore-crystal complexes were used in the bioassays.

Oligonucleotide PCR primers. Two types of novel primers were designed, namely, general primers (gen) from conserved regions of related *cry* genes and specific primers (spe) from highly variable regions, by using multiple alignments of reported DNA sequences (Table 2) using ClustalW and GeneWorks 2.3 (Intelligenetics, Inc.) (5). Oligonucleotides were synthesized in a DNA synthesizer (Microsyn 1450A; Systec Inc.) as specified by the manufacturer.

Sample preparation and PCR. *B. thuringiensis* strains were grown for 12 h on a nutrient medium plate. A loopful of cells was transferred to 0.1 ml of H_2O, frozen for 20 min at −70°C, and boiled for 10 min in water to lyse the cells. Cells were briefly spun (10 s at 10,000 rpm in an Eppendorf 5415C centrifuge), and 15 μl of supernatant was used as DNA template in the PCR. PCR mixtures were prepared as described previously (2, 3), and PCR was carried out in a Perkin-Elmer 480 thermal cycler as follows: 2 min at 95°C; 30 cycles of 95°C for 1 min, annealing (Table 2) for 1 min, and 72°C for 1 min; and 5 min at 72°C. A 15-μl sample was electrophoresed on 2% agarose gel.

Sequence of PCR products. PCR products were purified from 1% agarose gels with a QIAquick gel extraction kit as described by the manufacturer (Qiagen, Valencia, Calif.). Purified fragments were sequenced in the facilities of Universidad Nacional Autonoma de Mexico by using the same primers as used for amplification (GenBank no. AY326510 and AY326511). The sequences were analyzed with BLAST and ClustalW (5; http://www.ncbi.nlm.nih.gov/BLAST/).

Plasmid patterns. *B. thuringiensis* strains were grown to an optical density at 600 nm of 0.8 in Spizizen medium (0.2% NH_4SO_4, 1.4% K_2HPO_4, 0.6% KH_2PO_4, 0.1% sodium citrate, 0.02% $MgSO_4$-$7H_2O$) with 0.5% glucose, 0.1% Casamino Acids (DIFCO) and 0.01% yeast extract. Cells were washed in TE (50 mM Tris, 10 mM EDTA [pH 7.8]) and incubated for 30 min at 37°C in 10 mg of lysozyme/ml in 0.5 M sucrose, 25 mM Tris, and 10 mM EDTA (pH 8.0). After 10 min at 4°C, lysis buffer (0.2 M NaOH, 1% sodium dodecyl sulfate [SDS]) was added and the mixture was incubated for 5 min at 4°C. A solution of 3 M sodium acetate, pH 4.8, was added and stored for 20 min at −20°C. Particles were centrifuged at 12,000 rpm for 20 min in a Sorvall SS34 centrifuge. Two volumes of ethanol were added, and the mixture was incubated for 20 min at −80°C to precipitate DNA. DNA was centrifuged as above, dissolved in distilled water, and visualized in 0.6% agarose gels.

Protein electrophoresis. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed as described previously (14). Concentrated spore-crystal suspensions on disruption buffer were boiled for 5 min. Protein standards were carbonic anhydrase (29 kDa), ovalbumin (45 kDa), bovine serum albumin (66.2 kDa), phosphorylase B (97.4 kDa), β-galactosidase (116.25 kDa), and myosin (205 kDa).

Scanning electron microscopy. Diluted suspensions of spore-crystal complexes were placed on aluminum stubs and air dried. Samples were coated with gold in a Fullam EMS-76 M evaporator for 9 min and examined and photographed with a Philips XL30-ESEM scanning electron microscope operating at a voltage of 10 to 15 kV at ×2,000 and ×18,000 magnifications.

Bioassays. Mosquitoes were reared at 28°C, 87% relative humidity, and a 12:12 dark/light photoperiod as follows: *C. quinquefasciatus* (Recife strain) at Centro de Pesquisas Aggeu Magalhães/FIOCRUZ in Brazil; *A. aegypti* (CINVESTAV strain) at CINVESTAV-Irapuato in Mexico and (CIB strain) at Corporación para Investigaciones Biologicas in Colombia; and *A. albimanus* (INSP strain) at Instituto Nacional de Salud Pública in Mexico.

Insecticidal activity of *B. thuringiensis* strains was screened on larvae of the three mosquito species as described previously (10). Twenty early fourth-instar larvae were placed in 100 ml of dechlorinated water. Ten concentrations (2 to 2,000 ng/ml) of the spore-crystal complex of each strain were added (four repetitions). The *B. thuringiensis* subsp. *israelensis* positive control was included in addition to a dechlorinated water control. Larvae were incubated at 28°C and examined after 24 h. The mean 50% lethal concentration was estimated by Probit analysis using statistical parameters (9).

RESULTS

The *B. thuringiensis* strain collections used in this study were previously characterized by different methodologies (2) (Table 1). The analysis included (i) SDS-PAGE of spore-crystal to determine the number and size of Cry proteins, (ii) enzyme-linked immunosorbent and Western blot assays using different polyclonal antisera to identify the Cry groups, (iii) PCR using reported primers to identify some *cry* genes, and (iv) bioassays against different insect species to identify active strains. *B. thuringiensis* strains active against mosquitoes were selected for further characterization. We focused on strains that had higher activity than *B. thuringiensis* subsp. *israelensis* or that showed similar activity to *B. thuringiensis* subsp. *israelensis* but a different array of Cry proteins.

Microscopic observation of the crystals. Figure 1A shows the scanning electron microscopy observation of the crystals produced by strains LBIT315, LBIT320, LBIT348, IB604, and 147-8906. These strains showed small ovoid crystal inclusions very similar to those found in *B. thuringiensis* subsp. *israelensis*. The diameter of the crystals was 0.9 to 1.1 μm.

Insecticidal activity. Table 3 shows the insecticidal activities of selected strains against *A. aegypti*, *C. quinquefasciatus*, and *A. albimanus*. Strains LBIT315, LBIT320, LBIT348, and IB604 showed threefold-higher activity against *A. aegypti* and twofold-higher activity against *C. quinquefasciatus* larvae when compared with the *B. thuringiensis* subsp. *israelensis* control. Finally, strain 147-8906 showed similar insecticidal activity against *A. aegypti* when compared with *B. thuringiensis* subsp. *israelensis* but showed lower activity against *C. quinquefasciatus* and *A. albimanus*.

SDS-PAGE and plasmid profile analysis. The SDS-PAGE of spore-crystal suspensions of selected strains is shown in Fig.

TABLE 2. Characteristics of general and specific primers for *cry1*, *cry5*, *cry8*, *cry9*, *cry11*, *cry12*, *cry13*, *cry14*, *cry21*, and *cyt* genes

Primer pair	Anneal temp (°C)	Sequence[b]	Positions[a]	Gene(s) recognized	Product size (bp)	GenBank accession no.
cyt1gral	52	5′ CCTCAATCAACAGCAAGGGTTATT (d), 5′ TGCAAACAGGACATTGTATGTGTAATT (r)	197–674	*cyt1Aa*	477	X03182
			85–565	*cyt1Ab*	480	X98793
			97–574	*cyt1Ba*	477	U37196
cyt2gral	50	5′ ATTACAAATTGCAAATGGTATTCC (d), 5′ TTTCAACATCCACAGTAATTTCAAATGC (r)	509–865	*cyt2Aa*	356	Z14147
			529–884	*cyt2Ba*	355	U52043
			649–1004	*cyt2Bb*	355	U82519
			196–551	*cyt2Ca*	355	AAK50455
cry2gral	50	5′ GAGTTTAATCGACAAGTAGATAATTT (d), 5′GGAAAAGAGAATATAAAAATGGCCAG (r)	531–1057	*cry2Aa*	526	M31738
			376–902	*cry2Ab*	526	M23724
			2500–3020	*cry2Ac*	520	X57252
			1041–1541	*cry2Ad*	500	AF200816
cry4Aspe	50	5′ TCAAAGATCATTTCAAAATTACATG (d)	1706–2165	*cry4aA*	459	Y00423
cry4Bspe		5′CGTTTTCAAGACCTAATAATATAATACC (d), 5′ CGGCTTGATCTATGTCATAATCTGT (r)	1868–2189	*cry4Ba*	321	X07423
cry10spe	51	5′ TCAATGCTCCATCCAATG (d), 5′ CTTGTATAGGCCTTCCTCCG (r)	978–1326	*cry10*	348	M12662
cry11gral	50	5′ CGCTTACAGGATGGATAGG (d), 5′ GCTGAAACGGCACGAATATAATA (r)	990–1332	*cry11Aa*	342	M31737
			1025–1368	*cry11Ba*	343	X86902
			1048–1400	*cry11Bb*	352	AF017416
cry17 + 27	47	5′ CATTGTTCTACTTGGTATAA (d), 5′ GATACAATTACATCTCCTCCTGTA (r)	645–1477	*cry17Aa*	832	X99478
			1306–2201	*cry27Aa*	895	AB023293
cry19 + 39	51	5′ AAGCTGCGAATCTGCATTTACTTTT (d), 5′ CTCATAATTTTCCGTCCATAAAT (r)	1332–1948	*cry19Aa*	616	Y07603
			1209–1840	*cry19Ba*	631	D88381
			597–1216	*cry39*	619	BAB72016
cry24 + 40	48	5′ TTATCAATGTTAAGGGATGC (d), 5′ ACTGGATCTGTGTATATTTTCCTAG (r)	595–899	*cry24Aa*	304	U88188
			593–959	*cry40Aa*	366	BAB72018
cry25esp	53	5′ GGCTTCTAGATCAGGAGATGG (d), 5′ CATCATAATCAGAGCGCAGG (r)	1146–1706	*cry25*	560	U88189
cry29esp	50	5′ TCAGCTCCAATAACTGGTG (d), 5′ GCATGTCATCCCCTTGTCTA (r)	897–1348	*cry29*	451	AJ251977
cry30esp	50	5′ AACTCACACATCCTCCATCG (d), 5′ ATCGGAAGGCAATCATTCG (r)	2479–2744	*cry30*	265	AJ251978
cry32gral	54	5′ TGGTCGGGAGAGAATGGATGGA (d), 5′ ATGTTTGCGACACCATTTTC (r)	2236–2913	*cry32Aa*	677	AY008143
			2338–3014	*cry32Ba*	676	BAB78601
			2254–2930	*cry32Ca*	676	BAB78602
			2218–2894	*cry32D*	676	BAB78603

[a] Position at 5′ end of direct and reverse primers for each PCR primer pair.
[b] d and r, direct and reverse primers, respectively.

1B. Strains LBIT315, LBIT320, LBIT348, and IB604 had a protein profile similar to that reported for *B. thuringiensis* subsp. *israelensis*, with major proteins of 130, 70, and 28 kDa (8). Strain 147-8906 had four different protein bands of 100, 75, 65, and 26 kDa. LBIT315, LBIT320, LBIT348, and IB604 strains also had plasmid profiles similar to that of *B. thuringiensis* subsp. *israelensis* (Fig. 1C). In contrast, strain 147-8906 displayed a different plasmid profile.

Identification of specific *cry* genes in the *B. thuringiensis* isolates. The PCR methodology described here utilized 27 primers to detect 17 different *cry* and *cyt* genes described to codify for proteins active against mosquitoes (Table 2). Figure 2A shows the PCR products obtained with control strains. HD1 strain yielded a PCR product only when amplified with the cry2-gen primers. In contrast, *B. thuringiensis* subsp. *israelensis* showed the expected PCR products with cry4-spe, cry11-gen, and cyt-gen primers. The *B. thuringiensis* subsp. *jegathesan* was used as a control for *cry19*, *cry24*, and *cry25* genes. *B. thuringiensis* subsp. *jegathesan* also showed the expected PCR products when amplified with cry11-gen and cyt2-gen primers.

Some *cry* and *cyt* genes were identified in the native strains

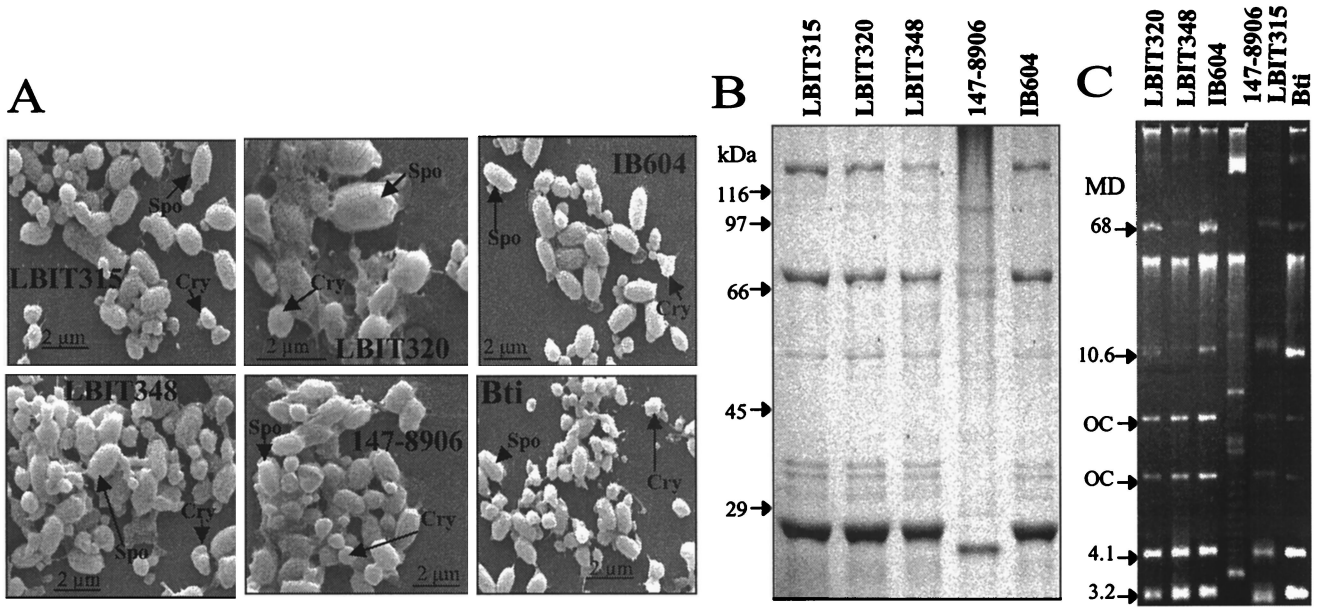

FIG. 1. Characterization of the selected *B. thuringiensis* strains. (A) Scanning electron microscopy observation of the spore and crystal mixtures produced by LBIT315, LBIT320, LBIT348, 147-8906, IB604, and *B. thuringiensis* subsp. *israelensis* HD567. Arrows point to crystal inclusions (Cry) and spores (Spo). (B) SDS-PAGE of spore-crystal suspensions of selected *B. thuringiensis* strains. (C) Agarose gel electrophoresis of the plasmid profile present in selected *B. thuringiensis* and *B. thuringiensis* subsp. *israelensis* HD567 strains. MD, megadaltons; OC, open circles.

(Table 3). Strain IB604 had the same *cry* and *cyt* genes present in *B. thuringiensis* subsp. *israelensis*. Strains LBIT315, LBIT320, and LBIT348 were similar to the *B. thuringiensis* subsp. *israelensis* strain with the exception of the *cry10* gene that was absent. Strain 147-8906 was positive with PCR primers of *cyt1*, *cyt2*, *cry11*, and *cry30* genes. However, this strain produced a PCR product with a different size than expected when assayed with the cry4B-spe primers (Fig. 2B).

Characterization of putative novel *cry* genes. The PCR product of strain 147-8906 obtained with cry4B-spe primers was sequenced (GenBank accession no. AY326511). BLAST analysis indicated that it corresponds to the *cry30* gene (100% identity). The direct primer hybridizes to the 3′region of this gene, and the reverse primer hybridizes outside of the coding region.

The PCR analysis performed with other *B. thuringiensis* strains that showed lower toxicity against mosquitoes than *B. thuringiensis* subsp. *israelensis* demonstrated that strain 447BrB produced a different size PCR product with the cry11-gen primers (Fig. 2B). The BLAST analysis of the sequence of this PCR product (GenBank no. AY326510) indicated that it corresponds to a new variant of *cry11B* (72% amino acid identity to Cry11Bb in the sequenced region). However, strain 447BrB presented low toxicity against the mosquito larvae and thus was not further characterized.

DISCUSSION

The great extension of Latin America, its different climatic regions, and diversity of insects provide the opportunity of isolating novel entomopathogenic bacteria. The *B. thuringiensis* strain analyzed in this report represents a sample of this diversity. We identified *B. thuringiensis* strains with higher activity against mosquitoes than the *B. thuringiensis* subsp. *israelensis* strain. Additionally, the PCR strategy described here could be useful for the characterization of other *B. thuringiensis* strain collections, as the novel primers could identify 17 different *cry* and *cyt* genes that codify for proteins active against mosquitoes.

B. thuringiensis strains that had higher activity than *B. thuringiensis* subsp. *israelensis* against *A. aegypti* and *C. quinque-*

TABLE 3. Dose-response insecticidal activities and *cry* and *cyt* gene profile present in the selected *B. thuringiensis* strains

Strain	50% lethal concn (ng/ml) (95% confidence interval) for:			*cry* and *cyt* gene profile
	A. aegypti	*C. quinquefasciatus*	*A. albimanus*	
LBIT315	4.12 (3.4–4.7)	7.21 (6.2–8.2)	31.17 (21.9–44.3)	*cry4A cry4B cry11 cyt1 cyt2*
LBIT320	4.16 (3.5–4.8)	11.85 (10.0–14.0)	19.15 (13.1–28.3)	*cry4A cry4B cry11 cyt1 cyt2*
IB604	4.23 (3.8–4.6)	7.52 (5.9–9.4)	14.51 (10.5–17.8)	*cry4A cry4B cry10 cry11 cyt1 cyt2*
LBIT348	5.08 (4.6–5.5)	6.61 (5.5–7.9)	12.13 (8.7–16.7)	*cry4A cry4B cry11 cyt1 cyt2*
147-8906	20.91 (18.7–23.1)	30.72 (23.2–51.3)	93.90 (76.7–130.6)	*cry11 cyt1 cyt2 cry30*
Bti[a]	13.86 (11.3–16.8)	17.52 (14.5–21.1)	21.15 (15.8–28.5)	*cry4A cry4B cry10 cry11 cyt1 cyt2*

[a] *B. thuringiensis* subsp. *israelensis*.

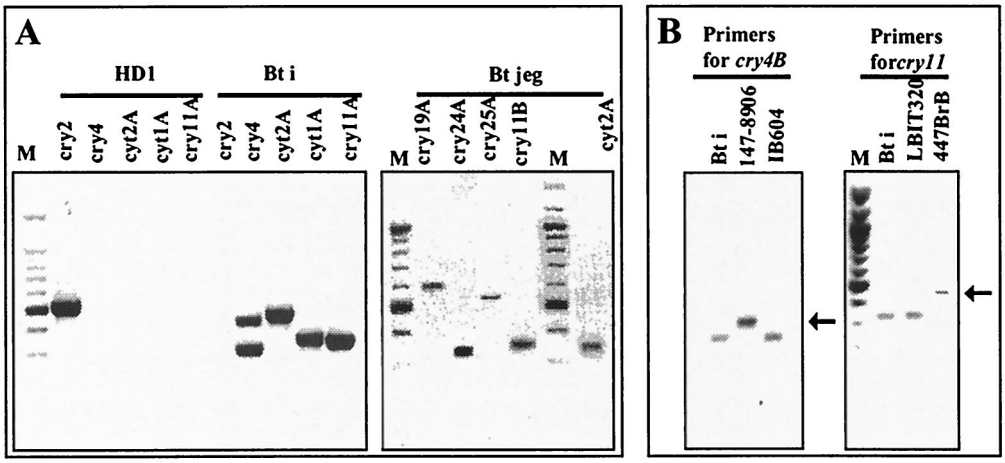

FIG. 2. (A) Agarose gel electrophoresis of the PCR products obtained with control strains HD1 *B. thuringiensis* subsp. *kurstaki*, HD567 *B. thuringiensis* subsp. *israelensis*, and *B. thuringiensis* subsp. *jegathesan*. (B) PCR products obtained with some native *B. thuringiensis* strains. Arrows point to PCR products that showed a different size than expected.

fasciatus larvae were identified in spite of their high similarity with this control strain (i.e., similar protein and plasmid profiles and similar *cry* and *cyt* genes). Other strains harboring other *cry* genes were also identified, but they showed lower activity against mosquitoes than *B. thuringiensis* subsp. *israelensis* (data not shown). These data support the idea that although a great variability in *cry* genes codifying for different mosquitocidal toxins exists in nature, one of the most effective combinations of proteins is that present in the *B. thuringiensis* subsp. *israelensis* strain, containing Cry4, Cry10, Cry11, and Cyt toxins (15). The higher mosquitocidal activity of the selected strains LBIT315, LBIT320, LBIT348, and IB604 could be due to different factors: the *cry* and *cyt* genes detected by PCR may represent fragments of genes encoding novel variants of known proteins; the *cry* and *cyt* genes may be identical, but the expression levels of at least some of them may be different; or an undetected factor or protein may be responsible for their higher activity. It will be worthwhile to isolate the *cry* and *cyt* genes of these strains to test the individual proteins and sequence these genes to identify differences responsible for the increased toxicity.

On the other hand, we identified an active strain, 147-8906, with different *cry* gene content than *B. thuringiensis* subsp. *israelensis* but with similar activity against *A. aegypti*. The proteins present in 147-8906 are all active against mosquitoes (S. Ordúz, personal communication) (http://www.biols.susx.ac.uk /Home/Neil_Crickmore/Bt/index.html). These data suggest that this strain may have a potential for the management of *B. thuringiensis* subsp. *israelensis* resistance in mosquito populations. Also, strain 147-8906 showed the lowest toxicity against *A. albimanus*, proving the possibility to select native strains that may be used in the biological control against some specific targets.

The results obtained with strain 447BrB suggest that it may harbor a putative novel *cry11B* gene. The cloning and expression of the whole gene and the characterization of its potential insecticidal activity against different mosquito species remain to be determined.

ACKNOWLEDGMENTS

We thank Lizbeth Cabrera, Regina Basurto, Eleazar Urbina, and Javier Luévano for technical assistance.

This work was supported in part by CYTED III.5, CONACYT Z001, USDA 2002-35302-12539, UC Mexus 2001, Colciencias 4501-07-532-97, and FAO.

REFERENCES

1. **Baird, J. K.** 2000. Resurgent malaria at the millennium: control strategies in crisis. Drugs **59:**719–743.
2. **Bravo, A., S. Sarabia, L. López, H. Ontiveros, C. Abarca, A. Ortiz, M. Ortiz, L. Lina, F. J. Villalobos, G. Peña, M.-E. Núñez-Valdez, M. Soberón, and R. Quintero.** 1998. Characterization of *cry* genes in a Mexican *Bacillus thuringiensis* strain collection. Appl. Environ. Microbiol. **64:**4965–4972.
3. **Cerón, J., L. Covarrubias, R. Quintero, A. Ortiz, M. Ortiz, E. Aranda, L. Lina, and A. Bravo.** 1994. PCR analysis of the *cryI* insecticidal crystal family genes from *Bacillus thuringiensis*. Appl. Environ. Microbiol. **60:**353–356.
4. **Chang, C., Y. M. Yu, S. M. Dai, S. K. Law, and S. S. Gill.** 1993. High-level *cryIVD* and *cytA* gene expression in *Bacillus thuringiensis* does not require the 20-kilodalton protein, and the coexpressed gene products are synergistic in their toxicity to mosquitoes. Appl. Environ. Microbiol. **59:**815–821.
5. **Devereux, J., P. Haeberli, and O. Smithies.** 1984. A comprehensive set of analysis programs for the VAX. Nucleic Acids Res. **12:**387–395.
6. **Guerchicoff, A., R. A. Ugalde, and C. P. Rubinstein.** 1997. Identification and characterization of a previously undescribed *cyt* gene in *Bacillus thuringiensis* subsp. *israelensis*. Appl. Environ. Microbiol. **63:**2716–2721.
7. **Guillet, P., D. C. Kurstack, B. Philippon, and R. Meyer.** 1990. Use of *Bacillus thuringiensis israelensis* for onchocerciasis control in West Africa, p. 187–190. *In* H. de Barjac and D. J. Sutherland (ed.), Bacterial control of mosquitoes and blackflies. Rutgers University Press, New Brunswick, N.J.
8. **Ibarra, J., and B. A. Federici.** 1986. Parasporal bodies of *Bacillus thuringiensis* subsp. *morrisoni* (PG-14) and *Bacillus thuringiensis subsp. israelensis* are similar in protein composition and toxicity. FEMS Microbiol. Lett. **34:**79–84.
9. **Ibarra, J. E., and B. A. Federici.** 1987. An alternative bioassay for determining the toxicity of suspended particles to mosquito larvae. J. Am. Mosq. Control Assoc. **3:**187–192.
10. **Mclaughlin, R. E., H. T. Dulmage, R. Alls, T. L. Couch, D. A. Dame, J. M. Hall, R. I. Rose, and P. L. Versoi.** 1983. U.S. standard bioassay for the potency assessment of *Bacillus thuringiensis* serotype H-14 against mosquito larvae. Bull. Ent. Soc. Amer. **30:**26–29.
11. **Regis, L., S. B. da Silva, and M. A. V. Melo-Santos.** 2000. The use of bacterial larvicides in mosquito and black fly control programs in Brazil. Mem. Inst. Oswaldo Cruz **95:**207–210.
12. **Regis, L., M. H. Silva-Filha, C. Nielsen-LeRoux, and J.-F., Charles.** 2001. Bacterial larvicides of dipteran disease vectors. Trends Parasitol. **17:**377–380.
13. **Restrepo, N., D. Gutierrez, M. M. Patiño, I. Thiery, A. Delecluse, and S. Ordúz.** 1997. Cloning, expression and toxicity of a mosquitocidal toxin gene from *Bacillus thuringiensis* subsp. *medellin*. Mem. Inst. Oswaldo Cruz **92:**257–262.

14. **Schägger, H., and G. von Jagow.** 1987. Tricine-sodium dodecylsulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. Anal. Biochem. **166:**368–379.
15. **Schnepf, E., N. Crickmore, J. Van Rie, L. Lereclus, J. Baum, J. Feitelson, D. R. Zeigler, and D. H. Dean.** 1998. *Bacillus thuringiensis* and its pesticidal crystal proteins. Microbiol. Mol. Biol. Rev. **62:**775–806.
16. **Travers, R. S., P. A. W. Martin, and C. F. Reichelderfer.** 1987. Selective process for efficient isolation of soil *Bacillus* sp. Appl. Environ. Microbiol. **53:**1263–1266.
17. **Wirth, M. C., G. P. Georghiou, and B. A. Federeci.** 1997. CytA enables CryIV endotoxins of *Bacillus thuringiensis* to overcome high levels of CryIV resistance in the mosquito, *Culex quinquefasciatus*. Proc. Natl. Acad. Sci. USA **94:**10536–10540.
18. **Wu, D., J. J. Johnson, and B. A. Federeci.** 1994. Synergism of mosquitocidal toxicity between CytA and CryIV proteins using inclusions produced from cloned genes of *Bacillus thuringiensis* subsp. *israelensis*. Mol. Microbiol. **13:** 965–972.
19. **Yu, Y. M., M. Ohba, and S. S. Gill.** 1991. Characterization of mosquitocidal activity of *Bacillus thuringiensis* subsp. *fukuokaensis* crystal proteins. Appl. Environ. Microbiol. **57:**1075–1081.